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Molecular characteristics and antimicrobial resistance in invasive and noninvasive Group B *Streptococcus* between 2008 and 2015 in China

Binghuai Lu [a,*,1], Xingchun Chen [b,1], Junrui Wang [c,1], Duochun Wang [d,e], Ji Zeng [f], Yi Li [g], Dong Li [g], Fengxia Zhu [a], Yanchao Cui [a], Lei Huang [h]

[a] Department of Laboratory Medicine, Civil Aviation General Hospital, Beijing 100123, China
[b] Department of Laboratory Medicine, People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, 530021, China
[c] Department of Clinical Laboratory, Affiliated Hospital of Inner Mongolia Medical University, Huhhot, 010050, China
[d] National Institute for Communicable Disease Control and Prevention, and State Key Laboratory for Infectious Disease Prevention and Control, Chinese Centre for Disease Control and Prevention, Beijing 102206, China
[e] Collaborative Innovation Centre for Diagnosis and Treatment of Infectious Diseases, Hangzhou, 310003, China
[f] Department of Laboratory Medicine, Wuhan Pu Ai Hospital of Huazhong University of Science and Technology, Wuhan, 430034, China
[g] Department of Laboratory Medicine, Henan Provincial People's Hospital, Zhengzhou, 450003, China
[h] Department of Laboratory Medicine, First Hospital, Peking University, Beijing, 100034, China

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ABSTRACT

Group B *streptococcus* (GBS) is an increasing pathogen threat to newborns and adults with immunodepressive diseases. Here, a total of 193 GBS, including 51 invasive and 142 noninvasive isolates, were collected from the patients with infections in 7 tertiary hospitals from 5 cities in China during the year 2008 to 2015. The strains of GBS were characterized by classical and molecular techniques for capsular polysaccharide serotyping, genes for pilus island (PI) and α-like protein (*alp*), and antibiotic resistance profiling. Of 193 isolates, the predominant serotypes were III (45.6%) and Ia (18.7%). All strains carried at least 1 PI gene. The combination of PI-2b and PI-1 was present in 46.1% isolates, followed by PI-2a alone (80, 41.5%) and PI-2b alone (23, 11.9%). The most prevalent *alp* gene was *rib* (87, 45.1%), followed by α-*C* (47, 24.4%), *ε* (33, 17.1%), *alp2/3* (7, 3.6%) and *alp4* (2, 1.0%), respectively. The clonal relationships between strains were investigated using multilocus sequence typing. The strains were distinguished into 26 individual sequence typing, and further clustered into 6 clonal complexes. A significant association was noted between the distributions of *alp* genes, serotyping and PI profiles, such as serotype III-*rib*-PI + PI-2a, Ib-α-*C*, and Ia-ε-PI-2a. No penicillin-resistant strains were detected, and 74.1%, 64.2%, and 68.9% were resistant to erythromycin, clindamycin, and tetracycline, respectively. The infective GBS isolates in China demonstrated epidemical features.

1. Introduction

Group B *Streptococcus* (GBS) is a common colonizer in both the genitourinary and gastrointestinal tract of 10–30% of asymptomatic woman, and the leading cause of neonatal infections (Verani et al., 2010). Besides pregnant women and neonates, recent documents showed that a remarkable increase in infections by GBS in adult and elderly patients with diabetes mellitus, cancer, renal dialysis, and other significant underlying diseases (Skoff et al., 2009; Teatero et al., 2015). The estimated mortality attributable to GBS severe infections in the elderly is ~15% (Chaiwarith et al., 2011; Edwards and Baker, 2005).

The polysaccharide capsule (*cps*) of GBS is a crucial virulence factor and stimulator of antibody-based immunity. Generally, the majority of GBS causing infections are serotype Ia, III, and V (Farley, 2001; Yoon et al., 2015). The data in the literature support that a conjugate vaccine with 5 serotypes (Ia, Ib, II, II, V) could potentially prevent more than 85% of global GBS (Melin and Efstratiou, 2013). Moreover, the proteins of 3 pilus island (PI) alleles, PI-1, PI-2a and PI-2b, were explored as potential virulence factors and promising vaccine targets (Margarit et al., 2009). A series of surface α-like proteins (*alp*) were demonstrated to play an important role in GBS pathogenesis and also considered as vaccine targets (Gilbert, 2004; Gravekamp et al., 1999). The *alp*s are encoded by genes of α-*C*, ε, *alp2/3*, *rib*, and *alp4*, respectively, whose distributions constitute the main epidemiological features of the local GBS isolates (Gilbert, 2004; Melin and Efstratiou, 2013). The epidemic features of PI and *alp* genes of infective GBS have been extensively reported in Africa, Europe, and America (Brimil et al., 2006; Gherardi et al., 2007; Madzivhandila et al., 2013; Margarit et al., 2009; Martins et al., 2013; Meehan et al., 2014; Shabayek et al., 2014); however, no data are available in China. Furthermore, erythromycin and clindamycin are

* Corresponding author. Tel.: +86-01085-762-244-2373.
E-mail address: zs25041@126.com (B. Lu).
[1] Contributed equally to this work.

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the drugs of choice for penicillin-hypersensitive patients with infections due to GBS; however, the raising nonsusceptible strains have been documented and caused global concern (Brimil et al., 2006; Gherardi et al., 2007; Wang et al., 2013). Predominant molecular characteristics of infective GBS strains differ geographically and over the time. Continuous monitoring of the epidemical changes is crucial for the treatment and prevention of clinical infections due to GBS. The external structures or surface molecules on GBS have been proposed as vaccine targets and explored (Madzivhandila et al., 2013). Development of a GBS vaccine needs detailed features of candidate antigens from geographically distinct, and temporally disparate GBS isolates, to determine the optimal vaccine formulation. Therefore, we initiate the current nation-wide epidemical investigation on GBS isolates causing infections, with the aim of demonstrating the prevalence and phenotypic and genotypic characteristics of infective GBS isolates collected from patients in China and compared our results with data reported worldwide.

2. Materials and Methods

2.1. Bacterial Isolates

A total of 193 GBS isolates responsible for clinical infections were included in this study. The patients enrolled were aged 18–89 years; 79.8% (154/193) were female and 71.5% (138/193) had underlying diseases, mainly diabetes mellitus (58 cases, 30.1%), renal disorder (34, 17.6%), and cancer (24, 12.4%). The strains under study were isolated from urine from urinary tract infections (n = 83), surgical-site, soft-tissue and other wound secretions (including ear tract infections, wound) (n = 49), bloodstream (n = 24), deep abscess including mastitis (n = 15), sputum and bronchoalveolar lavage fluid from lower respiratory infections (n = 9), fetal membranes from intrauterine infection (n = 6), aminiotic fluid (n = 3), cerebrospinal fluid (CSF) (n = 2), prostatic fluid from acute bacterial prostatitis (n = 1), and pleural effusion (n = 1). The patients admitted to the tertiary hospitals in China during 2008 to 2015 included: Civil Aviation General Hospital (CAGH) (Beijing) during the period 2008 to 2015, Peking University First Hospital (Beijing) during the period 2009 to 2014, Beijing Tongren Hosptial (Beijing) during 2012, Affiliated Hospital of Inner Mongolia Medical University (Huhhot) during the period 2008 to 2014, Henan Provincial People's Hospital (Zhengzhou) during the period 2014 to 2015, Wuhan Pu Ai Hospital of Huazhong University of Science and Technology (Wuhan) during the period of 2013 to 2015, and People's Hospital of Guangxi Zhuang Autonomous Region (Nanning) during the period of 2012 to 2015. The isolates were sent to the Department of Clinical Microbiology of CAGH for further confirmation by Christie–Atkins–Munch-Petersen test and 16S rRNA gene sequencing.

The invasive infection by GBS is defined as isolation of GBS from a commonly sterile site (bloodstream, abscess aspirate, sterile body fluids including CSF, aminiotic fluid, synovial, peritoneal, or pleural specimens etc.) with a compatible clinical syndrome. Otherwise the isolates were regarded as noninvasive.

2.2. Determination of cps Serotyping, PI Genes, and alp Genes Profile of GBS Isolates

The *cps* types were determined by amplifying *cps* type-specific regions of serotypes Ia, Ib, and II through IX (Yao et al., 2013), and the isolates that failed to type were deemed nontypeable (NT). Polymerase chain reaction (PCR) assays were used to identify the PI and surface *alp* genes profiling of the *alp* family using primer pairs described previously (Creti et al., 2004; Madzivhandila et al., 2013), and strains that tested negative for any of the known *alp* genes were considered negative.

2.3. Multilocus Sequence Typing, Phylogenetic and Epidemiological Analyses

Multilocus sequence typing (MLST) was analyzed by amplifying and sequencing 7 housekeeping genes as described elsewhere (Jones et al., 2003). Then, the sequence types (STs) and allelic profiles were confirmed by querying the MLST database (http://pubmlst.org/sagalactiae). The GBS isolates were assigned to 1 colonal complex (CC) if they shared 5 or more alleles with the predominant ST. The ST not identified in any cluster was assigned as singleton. BioNumerics software version 5.1 (Applied Maths, Belgium) was used to create minimum spanning trees to illustrate the relationships between MLST, CCs, PIs, and *alp* genes.

2.4. Antibiotic Susceptibility Testing and Macrolide Resistance Phenotypes

All isolates were tested for susceptibility to penicillin G, ampicillin, cefotaxime, erythromycin, clindamycin, and vancomycin by broth microdilution method (Tianjing Jin Zhang Science and Technology Development, China), and results were interpreted according to the breakpoints set for *Streptococcus* spp. β-Hemolytic group by the Clinical and Laboratory Standards Institute (CLSI, 2015). The macrolide-resistant isolates were further classified as constitutive macrolide, lincosamide, and streptogramin B (cMLSB) for those with erythromycin and clindamycin resistance, as inducible resistance (iMLSB), or M phenotype (macrolide-streptogramin B resistance and lincosamide susceptibility). Genetic determinants of *erm*(A) (subclass *erm*[TR]), *erm*(B), and *mef*(A/E) were investigated by PCR in erythromycin-resistant isolates (Gygax et al., 2006). The *linB* gene in the strains resistant to clindamycin but susceptible to erythromycin (L phenotype), was amplified using previously described primers (Gygax et al., 2006). Furthermore, the resistance genes of tetracycline, including *tet*(M), *tet*(K), *tet*(L) and *tet*(O), were detected using PCR as previously described (Ng et al., 2001).

3. Results

3.1. Infection Sources and cps Distribution

A total of 193 GBS isolates, invasive (51, 26.4%) or noninvasive, collected from 7 tertiary hospitals in China, were enrolled. Overall, the most common serogroups were III (88, 45.6%), Ia (36, 18.7%), Ib (30, 15.5%), and V (27, 14.0%), whereas II (5, 2.6%) and VI (3, 1.6%) were rarely discovered. Four isolates were NT. No strain was found to contain serotypes IV, VII, VIII or IX. Notably, *cps* V accounted for 19.6% (10/51) and 12.0% (17/142) of invasive and noninvasive population; however, *cps* Ia of invasive strains (6/51, 11.8%) were much lower than that of noninvasive ones (30/142, 21.1%). The detailed serotype distribution results are presented in Fig. 1.

Fig. 1. Correlation between CCs, ST, serotyping, *alp* genes, PIs, sampling sources, and of the Group B streptococcus (GBS) isolates. Minimum spanning tree analysis of GBS isolates according to ST, demonstrating the relationships between 193 infective GBS carrier isolates collected from 7 hospital settings of 5 cities. Note: in the minimum spanning tree, the STs are displayed as circles. The size of each circle indicates the number of isolates within this particular type. The founder ST was defined as the ST with the greatest number of single-locus variants. Serotyping (a), *alp* genes (b), PIs (c), and sampling sources profiling (d) are represented by different colors in the upper and down figures, respectively. STs that vary by 1 allele in their multilocus sequence typing profiles (single locus variants) are arranged in circles around the primary founder ST. Heavy solid lines represent single-locus variants; light solid lines represent double-locus variants; heavy dotted lines represent triple-locus variants; light dotted lines represent quadruple-locus variants.